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# The role of chromatin modifications in progression through mouse meiotic prophase

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Abbreviations: SC, synaptonemal complex; DSB, double strand breaks; PRC, polycomb repressive complex; MSUC, meiotic silencing of unsynapsed chromatin; MSCI, meiotic sex chromosome inactivation; PCH, pericentromeric heterochromatin; 5mC, 5-methylcytosine; UbH2A, ubiquitinated histone H2A;  $\gamma$ H2AX, phosphorylated histone H2AX; H3K27me3, histone H3 lysine 27 tri-methylation; H3K4me3, histone H3 lysine 4 tri-methylation; H3K9me1/2/3, histone H3 lysine 9 mono-/di-/tri-methylation

**Keywords: mouse; meiosis; chromatin; chromosome; histone modification; DNA methylation**

**Word count: 5100**

## Abstract

Meiosis is a key event in gametogenesis that generates new combinations of genetic information and is required to reduce the chromosome content of the gametes. Meiotic chromosomes undergo a number of specialised events during prophase to allow meiotic recombination, homologous chromosome synapsis and reductional chromosome segregation to occur. In mammalian cells, DNA physically associates with histones to form chromatin, which can be modified by methylation, phosphorylation, ubiquitination and acetylation to help regulate higher order chromatin structure, gene expression, and chromosome organisation. Recent studies have identified some of the enzymes responsible for generating chromatin modifications in meiotic mammalian cells, and shown that these chromatin modifying enzymes are required for key meiosis-specific events that occur during meiotic prophase. This review will discuss the role of chromatin modifications in meiotic recombination, homologous chromosome synapsis and regulation of meiotic gene expression in mammals.

## 25 Introduction

Meiosis is a specialised cell division involving a single round of DNA replication followed by two rounds of chromosome segregation to produce haploid gametes. During meiosis, chromosomes form a meiosis-specific organisational structure known as the synaptonemal complex (SC), and undergo homologous recombination to enable the pairing and subsequent segregation of homologous chromosomes (Cohen et al., 2006; Handel and Schimenti, 2010; Ollinger et al., 2010). The progressive formation of the SC is used to cytologically define the substages of meiotic prophase during which recombination takes place (Figure 1). SC formation begins in leptotene with short filaments of proteinaceous axial element assembling along each homologous chromosome, providing anchorage points for chromatin loops. Recombination also begins in leptotene with the generation of hundreds of targeted DNA double strand breaks (DSBs) across the genome. DSBs recruit recombination proteins such as RAD51 and DMC1 which promote a search for the chromosome's homologous partner to repair the damage. The axial element extends across the length of each chromosome axis and the homology search brings homologous chromosomes into close proximity stimulating synapsis in zygotene stage nuclei. Synapsis spreads along the axis of all paired chromosomes with the exception of the X and Y sex chromosomes in male spermatocytes which only pair in their region of homology, the pseudoautosomal region. Complete synapsis is characteristic of pachytene nuclei. DSBs progressively mature by association with recombination machinery and are repaired through prophase. Repair of a sub-population of DSBs generates crossovers in late pachytene which physically link homologous chromosomes together after the SC

45 disassembles during diplotene. Crossover interference helps distribute the crossovers between  
chromosomes so that every chromosome pair has at least one (Cohen et al., 2006; Handel and  
Schimenti, 2010; Ollinger et al., 2010).

The chromosomes in meiotic cells, like those in mitotic cells, comprises a complex of DNA  
50 and protein known as chromatin. The basic structural unit of chromatin is the nucleosome:  
approximately 200 base pairs of DNA wound round a central octamer of the core histones H2A,  
H2B, H3 and H4 (Figure 2). Histone H1, a linker histone, can associate with the core nucleosomes  
to mediate their organisation into higher order structures with different levels of compaction  
(Bannister and Kouzarides, 2011; Musselman et al., 2012; Zentner and Henikoff, 2013). Chromatin  
55 can be modified by methylation of the DNA, and methylation, acetylation, ubiquitylation and  
phosphorylation of the core histones. Chromatin modifications are generated and removed by  
specific ‘writer’ and ‘eraser’ enzymes, and these modifications can influence chromatin function by  
directly altering its structure and by recruiting ‘reader’ proteins that recognise these modifications.  
Chromatin modifications play important roles in the regulation of gene expression, chromosome  
60 compaction and organisation, and DNA repair in mitotic cells (Bannister and Kouzarides, 2011;  
Cedar and Bergman, 2012; Deaton and Bird, 2011; Musselman et al., 2012; Zentner and Henikoff,  
2013), and there is also growing evidence to suggest these modifications play similar roles in  
meiosis. Here we will review the role of chromatin modifications, and the proteins that read, write  
and erase those modifications, during meiotic prophase in mice.

65

## **Polycomb Repressive Complexes and Meiotic Entry**

Polycomb repressive complexes (PRCs) are one of the major regulators of gene expression  
in mammalian cells, binding target gene loci to induce specific histone modifications, chromatin  
compaction and transcriptional repression (Simon and Kingston, 2013). PRC1 and PRC2 catalyse  
70 the mono-ubiquitination of histone H2A at lysine 119 (UbH2A) and the tri-methylation of histone  
H3 at lysine 27 (H3K27me3) respectively (Cao et al., 2005, 2002; Simon and Kingston, 2013).  
Canonical repression through PRC1 and PRC2 is thought to involve initial recruitment of PRC2 to  
target loci, which then induces H3K27me3 at these sites. PRC1 is recruited by H3K27me3, which  
in turn induces UbH2A (Simon and Kingston, 2013). Hence, PRC1 is both a reader and writer of  
75 chromatin modifications. At least for some target genes PRC1-dependent chromatin compaction and  
repression of gene expression does not depend on its ability to ubiquitinate histones (Endoh et al.,  
2012; Eskeland et al., 2010), suggesting that PRC1 may be capable of repressing gene expression  
directly. In addition to canonical PRC repression, PRC1 and PRC2 can act independently to repress  
transcription at some loci (Leeb et al., 2010; Tavares et al., 2012). PRC-mediated repression of

80 transcription is not restricted to single copy genes, and extends to multi-copy retrotransposons (Leeb et al., 2010; Reichmann et al., 2012).

One group of genes that appears to be regulated by PRC-mediated repression in the developing germline is involved in regulating entry to meiosis. Conditional deletion of one of the catalytic ubiquitin ligase components of PRC1, *Rnf2* (also known as *Ring1B*), in XX primordial germ cells results in a global reduction in UbH2A levels and upregulation of a cohort of genes associated with early meiotic prophase (Yokobayashi et al., 2013). These include meiosis-specific cohesin subunits, synaptonemal complex components and *Stra8*, a gene required for entry into meiosis (Baltus et al., 2006). The *Stra8* promoter is physically associated with RNF2 and enriched for H3K27me3 in wild type primordial germ cells at E11.5, and *Stra8* expression is prematurely activated in *Rnf2*<sup>-/-</sup> XX germ cells at this stage. Together this suggests that *Stra8* is a direct target of canonical PRC-mediated repression in pre-meiotic germ cells in female embryos (Yokobayashi et al., 2013). The premature activation of *Stra8* in *Rnf2*-conditional knockout XX germ cells is accompanied by premature entry into meiosis. These mutant oocytes assemble synaptonemal complex but typically do not complete meiosis and die (Yokobayashi et al., 2013). Therefore PRC1 plays a role in regulating meiotic gene expression and entry into meiosis in female embryos (Figure 1).

PRCs may also have later roles in meiosis to facilitate chromosome synapsis during zygotene. Deletion of the core PRC1 component, *Cbx2* (also known as *M33*), results in significant germ cell loss in females from some strains of inbred mice (Baumann and De La Fuente, 2011). These mutant oocytes feature some chromosome asynapsis which appears to trigger a meiotic silencing of unsynapsed chromatin response (Baumann and De La Fuente, 2011) (Figure 1). It is not clear if these synapsis defects represent a direct function for PRCs in meiotic chromosome structure or synapsis, or changes in meiotic gene expression. Further analysis of chromosome synapsis and progression through meiotic prophase in mice carrying mutations in *Rnf2* and other PRC components would therefore be of interest.

## DNA Demethylation and Meiotic Gene Expression

110 DNA methylation, which primarily occurs at cytosine residues of CpG dinucleotides, is one of the most abundant chromatin modifications present in mammalian genomes (Cedar and Bergman, 2012; Deaton and Bird, 2011; Reddington et al., 2013a). DNA methylation at gene promoters is associated with transcriptional repression, although this association is affected by the density of CpG dinucleotides. Promoters with a high density of CpGs tend to be unmethylated and

115 transcriptionally active, while promoters with a low density of CpGs are typically methylated  
regardless of whether they are transcriptionally active or not. The main class of promoters where  
methylation is associated with transcription have an intermediate density of CpGs and tend to be  
methylated and repressed in a tissue-specific manner (Meissner et al., 2008; Weber et al., 2007).  
DNA methylation could potentially act to repress transcription by recruiting 5-methylcytosine  
120 (5mC) reader proteins (Hendrich and Bird, 1998; Lewis et al., 1992; Prokhortchouk et al., 2001) or  
by directly obstructing the binding of transcription factors to the DNA (Wiench et al., 2011). In  
general, DNA methylation is recruited to promoters that have already been silenced by other  
mechanisms, and acts as a secondary modification to stabilise the repressed state (Deaton and Bird,  
2011). However, a small group of genes have been identified that appear to use DNA methylation as  
125 a primary mechanism to silence expression (Hackett et al., 2012). During their migration to the  
developing gonad primordial germ cells initiate a phase of global demethylation that activates the  
expression of many of these methylation-sensitive genes prior to meiotic entry (Hackett et al., 2012;  
Hajkova et al., 2002; Seki et al., 2005). Genes involved in suppressing retrotransposons are highly  
enriched within this group of methylation-sensitive genes, and their co-ordinate regulation by DNA  
130 methylation might represent a developmental coupling between expression of germline genome  
defence mechanisms and the potential for retrotransposon activity in multiple hypomethylated cell  
types (Hackett et al., 2012; Reichmann et al., 2013). Many of these methylation-sensitive germline  
genome-defence genes are required for progression through meiotic prophase (Crichton et al.,  
2013). Setting up an appropriate DNA methylation status in the developing germline appears to be a  
135 prerequisite to allow appropriate gene expression for progression through meiotic prophase.

Multiple pathways are likely to be involved in the global demethylation event that occurs in  
the developing primordial germ cells (Hackett and Surani, 2013). One group of enzymes that are  
strongly linked to DNA demethylation is a family of three related TET DNA methyl dioxygenases  
140 which oxidise 5mC to generate 5-hydroxymethylcytosine, 5-formylcytosine, and 5-  
carboxylcytosine (Ito et al., 2011), which can then be actively or passively removed from the DNA  
(Hackett and Surani, 2013). Only *Tet1* and *Tet2* are expressed during the phase of global germline  
demethylation, and double knockdown of both these genes impairs demethylation in an *in vitro*-  
derived germ cell model (Hackett et al., 2013). A homozygous gene-trap mutation in *Tet1*, which  
145 fuses its DNA-binding CxxC domain to  $\beta$ -geo, reduces 5-hydroxymethylcytosine levels in E14.5  
germ cells and decreases fertility of both male and female mice (Yamaguchi et al., 2012). *Tet1* gene-  
trap mutant testes are morphologically normal and display no obvious germ-cell defects. However,  
female *Tet1* gene-trap mutants have fewer oocytes due to apoptosis at late foetal stages. Pachytene  
chromosomes in the *Tet1* mutant often fail to properly synapse and the number of oocytes

150 progressing to diplotene is greatly reduced (Yamaguchi et al., 2012). Although there is not a dramatic increase in global DNA methylation in the *Tet1* gene-trap mutant germ cells, assessment of gene expression at E13.5 when oocytes are entering meiotic prophase has revealed altered expression of over 1000 genes. Some of these misregulated genes, such as *Stra8*, *Sycp3*, *Mael*, and *Prdm9*, are required for meiosis, although not all of these genes have increased levels of DNA  
155 methylation at their promoters (Yamaguchi et al., 2012). It remains unclear which expression changes, if any, are responsible for the meiotic disturbance observed in *Tet1* gene-trap mutant germ cells and it will be important to establish whether *Tet1*-dependent demethylation is coordinating activation of a meiotic gene expression program in the developing germline.

## 160 DNA Methylation and Asynapsis

Following global erasure of DNA methylation in developing germ cells new methylation marks are established, at least some of which are sex-specific. Timing of *de novo* methylation differs dramatically between spermatogenesis and oogenesis: *de novo* methylation in male germ cells occurs in late foetal development, long before meiosis is initiated; whereas in females *de novo*  
165 methylation occurs during postnatal oocyte growth while the oocyte is arrested in the dictyate stage of meiotic prophase. Thus the global level of DNA methylation during early meiotic prophase differs with gender.

*De novo* methylation marks are written onto DNA by the DNA methyltransferases  
170 DNMT3A and DNMT3B, which have partially overlapping target loci (Kaneda et al., 2004; Kato et al., 2007; Okano et al., 1999). Once established, DNA methylation marks are then propagated by the maintenance DNA methyltransferase DNMT1 (Cedar and Bergman, 2012). Although mutations in either *Dnmt3a* or *Dnmt3b* result in hypomethylation at specific imprinted genes and/or repetitive sequences in spermatocytes and oocytes, these defects do not impair progression through meiosis  
175 (Kaneda et al., 2004; Kato et al., 2007; Yaman and Grandjean, 2006). DNMT3A and DNMT3B both interact with the partially homologous but catalytically dead protein, DNMT3L (Hata et al., 2002), which stimulates their activity (Suetake et al., 2004). The absence of DNMT3L results in reduced *de novo* DNA methylation at redundant target loci unaffected by the absence of DNMT3A or DNMT3B alone, including LINE-1 and IAP retrotransposons in prospermatogonia (Bourc'his and  
180 Bestor, 2004). Expression of LINE-1 and IAP is greatly increased in prospermatogonia and male *Dnmt3l*<sup>-/-</sup> mice display delayed entry into meiosis and abnormal meiotic progression. The *Dnmt3l*<sup>-/-</sup> spermatocytes are defective in synaptonemal complex formation, with widespread asynapsis and non-homologous synapsis resulting in failure to progress to the full pachytene stage (Bourc'his and Bestor, 2004). Recombination proteins RAD51 and RPA successfully localise to chromosome axis-

185 associated foci in *Dnmt3l*<sup>-/-</sup> spermatocytes, indicating that the synapsis defects are not the result of failure to initiate meiotic recombination (Mahadevaiah et al., 2008). Rather, asynapsis in *Dnmt3l*<sup>-/-</sup> spermatocytes appears to represent the impaired ability of meiotic chromosomes to successfully search for or recognise their homologous partner.

190 A number of other mouse mutants with DNA methylation defects also suffer defective chromosome synapsis and meiotic prophase arrest in spermatocytes. These include mutations in *Lsh* (also known as *Hells*), a putative accessory factor for *de novo* DNA methyltransferases, and *Miwi2* (also known as *Piwi4*), a piRNA-binding protein required for *de novo* DNA methylation in the male germline (Carmell et al., 2007; Myant and Stancheva, 2008; Zeng et al., 2011). Therefore the  
195 meiotic progression defects in *Dnmt3l*<sup>-/-</sup> spermatocytes are likely to be due to altered DNA methylation. The changes in DNA methylation in *Dnmt3l*<sup>-/-</sup> spermatocytes are also associated with some changes in histone modifications. Global levels of histone H4 acetylation and histone H3 acetylation, which are associated with active chromatin, normally decrease during early meiotic prophase in wild-type spermatocytes. However, this decrease is delayed in *Dnmt3l*<sup>-/-</sup> spermatocytes  
200 with levels of acetylated H3 and H4 remaining high in zygotene (Webster et al., 2005). The opposite change was observed for H3K9me2, a modification associated with transcriptional repression, which is globally reduced in zygotene *Dnmt3l*<sup>-/-</sup> spermatocytes (Webster et al., 2005). Together these changes in histone modifications indicate that globally there is more decompacted, transcriptionally active or permissive chromatin present in zygotene *Dnmt3l*<sup>-/-</sup> spermatocytes. It is not currently clear  
205 whether the chromosome asynapsis and meiotic arrest of *Dnmt3l*<sup>-/-</sup> spermatocytes is the result of transcriptional de-repression of retrotransposons, aberrant regulation of meiotic gene expression, or changes in chromatin structure perturbing of homologous chromosome synapsis (Crichton et al., 2013).

210 In contrast to spermatocytes, DNMT3L does not function until after recombination and synapsis is complete in oocytes, and is not required for these meiotic events (Bourc'his et al., 2001; La Salle et al., 2004). Although oocytes are globally hypomethylated compared to spermatocytes while they progress through early meiotic prophase, the DNA methylation that is present in meiotic oocytes does seem to play an important role in allowing meiotic prophase to proceed normally. *Lsh*  
215 is required for DNA methylation at repetitive elements and some single copy genes during development (Dennis et al., 2001; Sun et al., 2004). DNA methylation is reduced at *IAP* retrotransposon sequences in *Lsh*<sup>-/-</sup> oocytes, and *IAP* expression is greatly increased in *Lsh*<sup>-/-</sup> ovaries (De La Fuente et al., 2006). *Lsh*<sup>-/-</sup> oocytes successfully recruit the early recombination protein RAD51 to chromosome axes indicating normal initiation of recombination, but synapsis of



220 homologous chromosomes is defective (De La Fuente et al., 2006), reminiscent of *Dnmt3l*<sup>-/-</sup> spermatocytes. Therefore it appears that DNA methylation is required for successful chromosome synapsis in oocyte prophase, and reducing DNA methylation levels is associated with defects in chromosome synapsis during meiosis in male and female germ cells (Figure 1).

## 225 **Histone Phosphorylation, Histone Ubiquitination, and Meiotic Sex Chromosome Inactivation**

Phosphorylation adds a significant negative charge to histones, potentially loosening their interactions with DNA by repelling the negatively charged phosphates of the DNA backbone (Bannister and Kouzarides, 2011; Musselman et al., 2012; Zentner and Henikoff, 2013). The histone  
230 variant H2AX (also known as H2AFX) is phosphorylated at serine 139 by the ATM and ATR kinases in response to DNA DSBs in both mitotic and meiotic cells (Bellani et al., 2005; Rogakou et al., 1998). Approximately 200-300 DSBs are generated during meiosis by the endonuclease SPO11, which induces phosphorylation of histone H2AX ( $\gamma$ H2AX) at these sites (Figure 1) (Baudat et al., 2000; Romanienko and Camerini-Otero, 2000). The chromatin surrounding DSBs also becomes  
235 hyperacetylated on histone H4, indicating that some remodelling of the DSB-associated chromatin is taking place (Buard et al., 2009). As meiotic recombination progresses the  $\gamma$ H2AX domains undergo dynamic changes, starting out as diffuse clouds surrounding autosomal axes in leptotene and ending up as discrete foci associated with the axes themselves as the DSBs are repaired in zygotene and pachytene (Chicheportiche et al., 2007; Mahadevaiah et al., 2001). Despite its  
240 association with meiotic DSBs,  $\gamma$ H2AX does not appear to be required for the normal progression of meiotic recombination as *H2AX*<sup>-/-</sup> female mice are fertile, and recombination and synapsis appear to progress normally on the autosomes of *H2AX*<sup>-/-</sup> spermatocytes (Celeste et al., 2002; Fernandez-Capetillo et al., 2003). There may be some redundancy between phosphorylation of the H2AX histone variant and phosphorylation of the core histones H2A and H2B, which largely co-localise  
245 with  $\gamma$ H2AX during meiosis (Baarends et al., 2007).

$\gamma$ H2AX continues to associate with the unsynapsed chromosome axes if there is a failure in chromosome synapsis as DSB repair using the homologous chromosome is not possible (Turner et al., 2005). Such asynapsed chromosome axes undergo transcriptional silencing known as meiotic  
250 silencing of unsynapsed chromatin (MSUC) (Turner et al., 2005). A related phenomenon known as meiotic sex chromosome inactivation (MSCI) also takes place at the partially asynapsed XY chromosomes in pachytene spermatocytes and is essential for progression through meiotic prophase (Turner et al., 2005). MSCI involves chromatin compaction and associated histone modifications taking place throughout the XY chromatin, resulting in exclusion of RNA polymerase II and

255 transcriptional repression (Turner, 2007).  $\gamma$ H2AX is enriched at the inactive X and Y chromosomes, which reside in a structure known as the sex body, from pachytene until the diplotene-metaphase transition in males (Mahadevaiah et al., 2001) (Figure 1). The  $\gamma$ H2AX reader MDC1 is also localised to the sex body and, like H2AX, is essential for MSCI (Celeste et al., 2002; Fernandez-Capetillo et al., 2003; Ichijima et al., 2011). The sex body also undergoes deacetylation of H3 and  
260 H4 in pachytene (Khalil et al., 2004), although it is not clear which deacetylase erases these modifications. The addition of acetyl groups to lysine residues neutralises the positive charge in histone tails, weakening the interactions between histones and DNA, and increasing DNA accessibility, decompaction, and often transcriptional activity (Bannister and Kouzarides, 2011; Musselman et al., 2012; Zentner and Henikoff, 2013). Therefore the deacetylation of XY chromatin  
265 is consistent with the condensation and transcriptional silencing of XY chromosomes.

Ubiquitin is a relatively large 76-amino acid modification that can be targeted to lysine residues in histone tails. Ubiquitination is likely to induce structural changes to chromatin that can potentially disrupt or create binding sites for chromatin-associated ‘reader’ proteins, and some types  
270 of poly-ubiquitination can target histones for degradation (Bannister and Kouzarides, 2011; Braun and Madhani, 2012; Cao and Yan, 2012). Mono-ubiquitination of histones H2A and H2B at lysine 119 and 120 respectively has been widely studied in mitosis in association with transcriptional regulation and DNA damage response (Braun and Madhani, 2012; Cao and Yan, 2012). UbH2B levels are very low in spermatocytes (Baarends et al., 2007) and the localisation pattern has not  
275 been reported. In contrast, UbH2A levels are readily detectable in meiosis, and localise to asynapsed axes in both spermatocytes and oocytes, consistent with a role in MSUC. UbH2A is also enriched at the sex body in pachytene spermatocytes, consistent with a role in MSCI (Baarends et al., 2005; Kouznetsova et al., 2009) (Figure 1). Ubiquitination of H2A at unsynapsed axes and the sex body occurs later than H2AX phosphorylation and dissociates from the sex body before the end  
280 of pachytene (Baarends et al., 2005). UbH2A is not detected in pachytene spermatocytes lacking RNF8 (Lu et al., 2010), indicating that this ubiquitin ligase is required for ubiquitination of H2A in this stage of meiotic prophase. Despite its association with transcriptional repression, the role of UbH2A on asynapsed chromosomal axes and XY chromatin is not clear as it is dispensable at the sex body for spermatogenic progression (Lu et al., 2010).

285

## Histone Methylation and Meiotic Recombination Hotspots

Histone methylation mainly occurs at arginine and lysine residues. In contrast to acetylation and phosphorylation, addition of a methyl group does not alter the charge of the histone and residues may become mono-, di- or tri-methylated (Bannister and Kouzarides, 2011; Musselman et

290 al., 2012; Zentner and Henikoff, 2013). Histone methylation is thought to function primarily through the recruitment of reader proteins that bind to methylated histones and locally influence transcription and chromatin structure (Bannister and Kouzarides, 2011; Musselman et al., 2012). Tri-methylation of histone H3 at lysine 4 (H3K4me3) is a histone modification typically associated with the transcription start site of active genes (Schneider et al., 2004). However, during meiosis  
295 H3K4me3 is additionally associated with genomic hotspots for meiotic DSBs (Buard et al., 2009), suggesting that this chromatin modification could play a role in recruiting the meiotic endonuclease SPO11 to these sites in the genome (Figure 1). The establishment of meiosis-specific H3K4me3 in mice is performed by the meiosis-specific histone methyltransferase PRDM9 (also known as MEIS1) (Brick et al., 2012; Hayashi et al., 2005). While there is a clear association between  
300 PRDM9-dependent H3K4me3 and meiotic DSB formation, it remains unclear how PRDM9-dependent H3K4me3 sites (~15% of the total H3K4me3 loci) are distinguished from the H3K4me3 at the transcription start site of active genes. It is possible that PRDM9 directly recruits SPO11 and any additional machinery necessary for DSB formation, or that these proteins are recruited by a combination of a H3K4me3 reader and the local chromatin environment. Changes in chromosome  
305 organisation are also likely to be important during the generation of meiotic DSBs to bring PRDM9-dependent H3K4me3 sites and SPO11 in chromatin loops into contact with components of the meiotic recombination machinery localised to the chromosome axis (Borde and de Massy, 2013). In the absence of PRDM9, meiotic DSBs relocate, often forming at active gene promoters which remain marked with PRDM9-independent H3K4me3 (Brick et al., 2012). Male and female  
310 mice lacking PRDM9 are infertile following germ cell loss in late prophase. *Prdm9*<sup>-/-</sup> germ cells have impaired chromosome synapsis and progression of meiotic recombination (Brick et al., 2012; Hayashi et al., 2005), but the mechanistic basis for the asynapsis remains unclear. Altered gene expression in *Prdm9*<sup>-/-</sup> spermatocytes has been proposed as a possible route via which meiosis could be perturbed (Hayashi et al., 2005). Alternatively the misplacement of meiotic DSBs has been  
315 proposed to result in an impaired homology search and defective progression through meiotic prophase I (Brick et al., 2012). Further work is required to determine if the methyltransferase ‘writer’ activity of PRDM9 is essential for its functions in recombination, and to understand whether specific readers of the H3K4me3 mark might play a role in recruiting the meiotic recombination machinery to recombination hotspots.

320

Although recombination hotspots are associated with H3K4me3 in mice, the DNA sequences located at these sites vary between different strains (Baudat et al., 2010; Brick et al., 2012; Buard et al., 2009; Smagulova et al., 2011). Rapid evolutionary divergence of the DNA-binding zinc-finger domain of *Prdm9* alleles is responsible for the strain-specific meiotic H3K4me3

325 and DSB profiles, demonstrating that PRDM9 is a master regulator of hotspot localisation (Brick et al., 2012; Grey et al., 2011). Strain-specific polymorphisms in *Prdm9* are also major contributors to hybrid sterility in mice, implicating meiosis-specific targeting of H3K4me3 in the process of speciation (Mihola et al., 2009). Therefore H3K4me3 can be targeted to strain-specific locations in meiosis while remaining associated with DSB formation, suggesting that a currently unknown  
330 H3K4me3-reader could link DNA sequence with the meiotic recombination machinery.

## Histone Methylation and Centromere Clustering

Histone H3 lysine 9 mono-, di-, and tri-methylation (H3K9me1/2/3) are largely repressive chromatin marks associated with heterochromatin formation and transcriptional silencing (Bannister et al., 2001; Barski et al., 2007). G9a (also known as KMT1C) is a histone H3 lysine 9 mono- and  
335 di-methyltransferase that plays an essential role in the germline of both male and female mice (Tachibana et al., 2007). Spermatocytes lacking G9a do not contain any detectable H3K9me1 or H3K9me2 in their nuclei, display defects in chromosome synapsis and undergo apoptosis in pachytene (Figure 1). Similar defects are also apparent in *G9a*<sup>-/-</sup> oocytes (Tachibana et al., 2007).  
340 Although some gene expression changes are reported in *G9a*<sup>-/-</sup> testes, these are minimal and not thought to be responsible for the meiotic defects (Tachibana et al., 2007). Rather, the mechanism by which *G9a* mutation perturbs chromosome synapsis might relate to the clustering of centromeres into heterochromatic domains (Takada et al., 2011). Spermatocytes lacking G9a demonstrate an increase in heterochromatic clusters of centromeres, with each cluster containing fewer  
345 centromeres. This failure to maintain centromeric proximity between potentially homologous chromosomes may retard chromosome pairing (Takada et al., 2011).

H3K9me3 and the heterochromatin-binding protein HP1 $\gamma$  (also known as CBX3) have also been proposed to play a role in meiotic centromere clustering (Takada et al., 2011). H3K9me3 is  
350 enriched at pericentromeric heterochromatin (PCH) throughout meiotic prophase due to the activity of multiple histone methyltransferases (Peters et al., 2001; Tachibana et al., 2007). The related SUV39H1 and SUV39H2 histone methyltransferases play redundant roles in methylating H3K9 at PCH in leptotene spermatocytes, and alternative histone methyltransferases make increasing contributions to H3K9 methylation at PCH as meiotic prophase proceeds (Peters et al., 2001). HP1 $\gamma$   
355 is a reader of H3K9me3 and localises to PCH throughout meiotic prophase in spermatocytes. Spermatocytes lacking either HP1 $\gamma$  or SUV39H1/2 also exhibit impaired chromosome synapsis and, at least for HP1 $\gamma$ -deficient spermatocytes, this asynapsis is associated with defective centromere clustering (Takada et al., 2011). SUV39H1/2 and HP1 $\gamma$  appear to be required to localise G9a to PCH and generate H3K9me2 at these sites in early meiotic prophase (Takada et al., 2011).

360 Therefore cross-talk between these chromatin modifications at PCH appears to be important for centromere clustering and chromosome synapsis in meiotic prophase.

## Histone Acetylation and Exit from Meiotic Prophase

Chromatin modifications, particularly histone acetylation, also play a role in late meiotic  
365 prophase. In addition to influencing the compaction of DNA, histone H4 can also act as a platform for binding bromodomain proteins as readers of this active chromatin mark. BRDT is a testis-specific bromodomain protein expressed from the onset of meiosis (Pivot-Pajot et al., 2003), and is known to bind hyperacetylated histone H4 (Sasaki et al., 2009). In the absence of BRDT, chromosome pairing, SC formation and sex body formation all proceed normally, however  
370 spermatocytes arrest at diplotene and don't perform the first meiotic division (Figure 1). BRDT is a major determinant of the testis-specific gene expression program, binding acetylated histones at transcriptional start sites and influencing the expression of thousands of genes in meiotic prophase. The meiotic arrest observed in *Brdt*<sup>-/-</sup> is attributed to the failed activation of cyclin A1 expression (Gaucher et al., 2012), which is essential for spermatocyte exit from prophase and progression to  
375 the first meiotic division (Nickerson et al., 2007). Thus the expression of specific chromatin modification readers during meiosis can play an important role in enabling chromatin modifications to function during meiotic prophase.

## Conclusions and Perspective

380 Chromatin modifications appear to play a key role in facilitating multiple aspects meiotic prophase in mice. Readers, writers and erasers of histone modifications all facilitate some of the key chromosomal events that occur in meiosis and contribute to progression through meiotic prophase (summarised in Table 1). Clearly there are significant gaps in the current state of knowledge in this field, and the meiotic readers, writers and erasers for many of the modifications we have described  
385 in this review remain to be identified (Table 1). Furthermore, there are numerous additional histone modifications, including sumoylation, glycosylation, deimination and ADP ribosylation (Bannister and Kouzarides, 2011; Musselman et al., 2012; Tan et al., 2011), which could potentially play a role in meiotic chromatin structure and function but whose roles in meiosis have not yet been studied. Understanding how chromatin is organised in meiotic cells, and how chromatin modifications  
390 influence and regulate that organisation, is likely to generate insights into the processes of meiotic recombination, homologous chromosome synapsis, and meiotic sex chromosome inactivation.

Although we have mainly discussed individual chromatin modifications separately in this review, there is significant cross-talk between different chromatin modifications in non-meiotic cells (Bannister and Kouzarides, 2011; Musselman et al., 2012). For example, cross-talk between DNA methylation and PRCs means that around a third of the genes that change expression in mouse fibroblasts carrying mutations in the DNA methylation machinery do so because they are targets for PRC-dependent repression (Reddington et al., 2013b). Cross-talk between different histone modifications could potentially help integrate different aspects of chromatin function in meiotic prophase and provide links between meiotic chromosome organisation, recombination, and synapsis. The availability of state-of-the-art methods and reagents for genome-wide mapping of DNA and histone modifications, in combination with expanding resources of readily available gene-trap and conditional knockout mouse models, is likely to generate further links between chromatin modifications and meiosis, and to improve our mechanistic understanding of how chromatin structure influences the key chromosomal events that characterise meiotic prophase.

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## 415 **Figure Legends**

### **Figure 1. Meiotic prophase and events requiring chromatin modifications.**

Replicated homologous chromosomes (light and dark blue) are held together during meiotic prophase. DNA double strand breaks (DSBs) are generated by the endonuclease SPO11 in leptotene (indicated by scissors) while the axial element of the synaptonemal complex (red) assembles on chromosomes. DSBs recruit recombination proteins (green foci) and homologous chromosomes  
420 begin to pair and synapse (red connections) in zygotene. Synapsis is completed in pachytene and DSBs repair, with some producing crossovers connecting homologous chromosomes in diplotene as the synaptonemal complex disassembles. Abbreviations for chromatin modifications are as described in the text; H4ac, histone H4 acetylation.

### 425 **Figure 2. Role of chromatin modifications in regulating chromatin structure and function.**

DNA (orange) is wound round histones (blue) to form nucleosomes. Two copies of each of the core histones H2A, H2B, H3 and H4 (light blue) are present in the core nucleosome, which can associate with the linker histone H1 (dark blue). Different post-translational modifications to the chromatin are associated with transcriptionally active, decompacted chromatin and transcriptionally repressed,  
430 compacted chromatin as indicated.

## Tables

Chromatin Modification	General Role	Meiotic Writers	Meiotic Erasers	Meiotic Readers	Meiotic Function
DNA methylation	Transcriptional repression	DNMT1 DNMT3A DNMT3B	TET1 TET2	?	Erasure of DNA methylation implicated in gene expression programmes essential for meiosis De novo DNA methylation implicated in chromosome synapsis
Histone H3 K27 tri-methylation	Transcriptional repression	PRC2	?	PRC1	Implicated in gene expression programme controlling entry into meiosis PRC1 implicated in chromosome synapsis
Histone H3 K4 tri-methylation	Transcriptional activation	PRDM9	?	?	Associated with meiotic recombination hotspots
Histone H3 K9 mono-/di-methylation	Transcriptional repression	G9a SUV39H1 SUV39H2	?	HP1 $\gamma$	Associated with centromeric clustering and chromosome synapsis
Histone H2AX phosphorylation ( $\gamma$ H2AX)	Induced at DNA double strand breaks	ATM ATR	?	MDC1	Associated with sex body formation and MSCI. Present at early meiotic recombination foci, but not essential for meiotic recombination
Histone H2A mono-ubiquitination	Transcriptional repression	RNF8 PRC1	?	?	RNF8-dependent UbH2A associated with, but not required for, sex body formation and MSCI PRC1 associated with gene expression programme controlling entry into meiosis
Histone H4 acetylation	Transcriptional activation	?	?	BRDT	Implicated in regulating gene expression during pachytene to allow exit from meiotic prophase

**Table 1. Summary of major chromatin modifications implicated in progression through**

**meiotic prophase.** Proteins involved in writing, reading and erasing chromatin modifications in

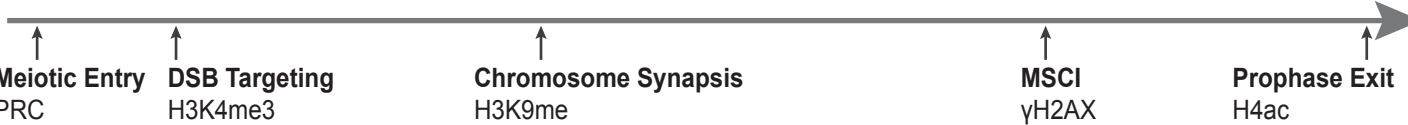
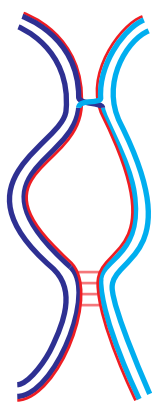
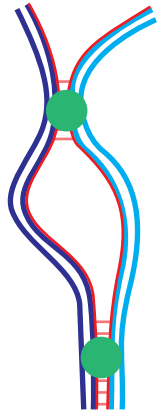
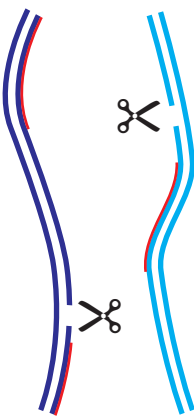
5 meiosis, or in pre-meiotic germ cells, are indicated. The potential meiotic functions of these chromatin modifications are summarised in the table. See text for details. For clarity, additional writers, readers, and erasers for these chromatin modifications operating in somatic or mitotic cells are not listed.

Leptotene

Zygotene

Pachytene

Diplotene



Meiotic Entry  
PRC

DSB Targeting  
H3K4me3

Chromosome Synapsis  
H3K9me  
PRC  
5mC

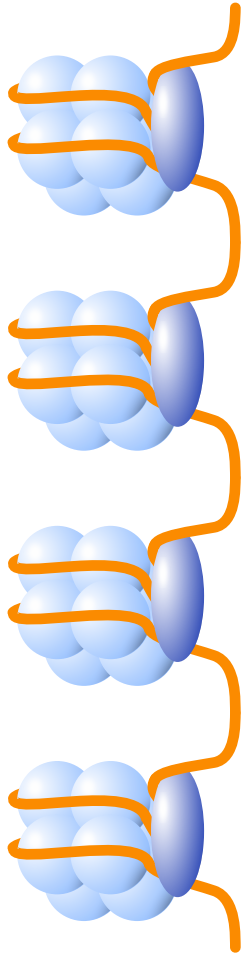
MSCI  
γH2AX  
UbH2A

Prophase Exit  
H4ac



Recombination  
γH2AX  
UbH2A

**Transcriptionally active /  
decompacted chromatin**



Histone H3K9 methylation  
Histone H3K27 tri-methylation  
Histone H2A ubiquitination  
Histone H2AX phosphorylation  
DNA methylation



Histone H3K4 tri-methylation  
Histone H3/H4 acetylation

**Transcriptionally repressed /  
compacted chromatin**

